

# Citrus Chemical Call to Arms Found



A *Diaprepes* weevil feeds on a citrus leaf.

When chewed on by hungry caterpillars, corn, cotton, and tobacco plants release chemical distress signals that marshal help from parasitic wasps. Similarly, lima beans attacked by spider mites attract predatory mites.

Now, an Agricultural Research Service-University of Florida team has shown that some citrus trees also resort to signaling when their roots are attacked by the grublike larvae of *Diaprepes abbreviatus*, the citrus root weevil. But instead of fast-flying wasps or nimble-legged predatory mites, the rescuers are wormlike organisms called “nematodes,” which wriggle inside the grubs and feed on them internally, killing the citrus pests in 24 to 48 hours.

“When weevil larvae feed on roots,” says ARS chemist Hans Alborn, “the roots release volatiles, including terpenes, that diffuse into the surrounding soil.” Nematodes in the soil track those chemical cues back to their source—namely, roots needing rescue from further harm.

Potentially, the finding could yield new, improved ways of using the nematodes to

biologically control *D. abbreviatus*. The species, which is native to the Caribbean region, was accidentally introduced into Florida in 1964. Today, it’s considered a major agricultural pest that causes \$70 million annually in losses not only to citrus, but also to ornamental plants and other crops.

The weevil also poses an aboveground threat to Florida’s citrus crop, valued at \$993 million during the 2008-2009 season. As an adult, the pest feeds on leaves, giving them a notched appearance. “Notching is one of the first things you see when entering an infested grove,” says Jared G. Ali, a University of Florida researcher collaborating with Alborn, who is in the ARS Chemistry Research Unit in Gainesville, Florida.

Adult weevils can be controlled by spraying the tree canopy with foliar insecticides. Also effective, though far more time-consuming and labor-intensive, is shaking the canopy and trapping the weevils that fall from trees or emerge from the soil.

Synthetic pyrethroids are used to control the grubs. Some of these chemicals are meant to serve as a barrier to larvae that fall to the ground after hatching from eggs deposited in the canopy. Even then, “many larvae still make it into the soil,” notes Ali, who works in the laboratory of Lukasz Stelinski, an assistant professor

ARS chemist Hans Alborn loads an autosampler with vials for gas chromatography and mass spectrometer analyses of root volatile collections.

## Could Improve Use of Nematode “First Responders”

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Above: University of Florida collaborator Jared Ali adds insect-killing nematodes to a six-arm olfactometer to test their attraction to different citrus rootstock cultivars attacked by *Diaprepes* root weevil larvae. The olfactometer is filled with sandy soil to simulate conditions below ground.



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at the University of Florida's Citrus Research and Education Center in Lake Alfred, Florida.

Once below ground, the grubs feed on the tree's fibrous roots until ready to pupate, emerging as adults anywhere from 6 to 15 months later. Severe infestations weaken the tree and reduce overall fruit yield. Feeding also increases the likelihood of infection by *Phytophthora* fungi, which cause root rots that can speed the tree's demise at great cost to growers.

### Biological Alternatives

Life below the citrus grove is not without its hazards, however. That's because the grubs are a favorite food of some species of insect-killing, or "entomopathogenic," nematodes such as *Steinernema riobrave* and *Heterorhabditis indica*. Both have been commercially formulated into biopesticide products that can be applied to grub-infested groves using existing herbicide applicator technology or other micro-sprinkler systems.

After encountering a grub, and infecting it, the nematodes release symbiotic bacteria that render the pest's tissues into a kind of slurry, which the nematodes then eat. Once the food is gone, the nematodes exit the carcass to start the cycle over again. The nematodes and their bacteria target a slew of insect hosts, but pose no danger to humans, pets, livestock, or wildlife.

A variety of factors, including soil type and temperature, can affect the nematode's performance as a biocontrol agent—with reductions in grub numbers at treated sites ranging from 0 to 90 percent. But until recently, little attention had been paid to the complex chemical tête-à-tête that occurs between citrus tree roots, grubs that feed on them, and the surrounding soil's resident nematodes.

When a plant chemically recruits a predator or parasite to dispatch of a herbivorous attacker—in this instance, citrus calling on nematodes to kill grubs—scientists call the phenomenon a "tritrophic interaction."



University of Florida assistant professor Lukasz Stelinski applies a nematode attractant to a nematode lure to be tested below ground in a citrus field.

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A chamber used in an underground field test of nematode attractants. The chamber is filled with sand, a root weevil larva, and a filter paper impregnated with the attractant.

While aboveground tritrophic interactions are well documented, far less is known about underground ones.

"We're one of the first groups to look at these interactions from a citrus-chemical ecology perspective," says Ali who coauthored a paper on the work with Stelinski and Alborn in the March 2010 issue of the *Journal of Chemical Ecology*. "Our specific focus is gaining insight into what a citrus plant can do to protect itself," Ali adds.

### Catching the Action in Real-Time

To determine what signaling compounds citrus roots released when attacked by grubs, the scientists used a specialized glass

chamber called a "root-zone olfactometer."

"With this system, you can see the weevils chewing on the roots and pull the volatiles off as they're being released," says Ali. "You can also tease out which direction the nematodes are going" and extract them for analysis, he adds.

In lab and greenhouse trials with small trees derived from the commercial rootstock "Swingle citrumelo," roots damaged by captive grubs attracted up to three times more *S. diaprepesi* nematodes than roots that had been mechanically damaged or left intact. When root volatiles were collected and analyzed by gas chromatography-mass spectrometry, terpenes accounted for four of the six compounds that stood out. Interestingly, "the roots only released the volatiles when being fed on by larvae," notes Alborn.

The team's research could one day lead to new varieties developed from rootstocks shown to be adept at recruiting nematodes. So far, they have tested the signaling capacities

of 5 rootstocks and hope to screen as many as 20 more by the end of 2011.

The researchers are also studying the volatiles' effect on other denizens of the citrus root zone, including nematodes that parasitize plants.

Within the next 2 years, the team hopes to recommend rootstock-nematode combinations that growers can use as part of an integrated approach to managing the weevil.—By **Jan Suszkiw**, ARS.

*This research is part of Crop Protection and Quarantine (#304) and Crop Production (#305), two ARS national programs described at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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